DEVELOPMENT OF A VACCINE TO PREVENT *FALCIPARUM* MALARIA INFECTION

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Abstract

Our research has resulted in the identification of a candidate *Plasmodium falciparum* malaria vaccine. The vaccine candidate is composed of a well-characterized neutralizing B cell epitope and a universal human T cell epitope, both derived from the circumsporozoite (CS) protein delivered on a highly immunogenic particulate carrier protein that effectively delivers 240 copies of each epitope on each particle. The carrier is an engineered derivative of the hepatitis B core protein (HBc), which is known to be immunogenic and safe in humans. Prototype vaccines have been shown to be 95% effective in protecting mice from *P. berghei* and *P. yoelii* challenge infections.

When formulated in alum, the vaccine candidate is a potent immunogen in mice eliciting unprecedented titers of sporozoite-neutralizing antibody. A universal CS-derived T cell epitope has been demonstrated to facilitate both a significant reduction in parasite load in the *P.yoelii* infected mice and effectively stimulates human T cells in vitro. The cell-mediated immunity afforded by the vaccine candidate should facilitate boosting of anti-sporozoite antibody titers following parasite exposure in endemic regions, as well as the direct targeting of parasite-infected hepatocytes.

The vaccine candidate has been produced in abundant quantities in *E.coli* using newly developed expression and purification methods that are fully scalable. Future work will focus on three areas: 1) scale-up of vaccine production for toxicology and clinical trials. 2) safety testing in animals and humans, and 3) efficacy testing in clinical trials in the U.S. and endemic areas.

Introduction: The Disease

Malaria is by far the world's most important tropical parasitic disease, and kills more people than any other communicable disease, with the exception of tuberculosis. The causative agents in humans are four species of *Plasmodium* protozoa: *P.falciparum*, *P.vivax*, *P.ovale* and *P.malariae*. Of these, *P.falciparum* accounts for the majority of infections and is the most lethal.

Malaria is a public health problem today in more than 90 countries, inhabited by a total of 2.4 billion people - 40% of the world's population.

- Worldwide prevalence of the disease is estimated to be on the order of 300-500 million clinical cases each year
- More than 90% of all malaria cases are in sub-Saharan Africa. Two-thirds of the remainder are concentrated in six countries, India, Brazil, Sri Lanka, Viet Nam, Columbia and Solomon Islands, in decreasing order of prevalence.
- Mortality due to malaria is estimated to be in the range of 1.5 to 2.7 million deaths each year, accounting for one person every 12 seconds. The vast majority of deaths occur among young children in Africa, especially in remote rural areas with poor access to health services.
- There are 7 million travelers from the U.S. each year to endemic areas. One source estimates that there are as many as 20 million arrivals from the U.S. into countries other than Africa, where malaria is prevalent (World Tourism Organization)
- The U.S. reports about 1,000 cases of malaria each year. About 10,000 cases are reported each year in Europe, alone.

(Data from , WHO Technical #92, December 1998, and IHI)

The Approach

The positive correlation between protection against malaria infection and anti-Circumsporozoite (CS) antibody titer has been demonstrated repeatedly over the past 15 years [2, 3]. The evidence that a vaccine which can elicit high-titer, long-lived antibody responses in sufficient vaccine recipients can be protective suggests that protection against malaria infection is achievable via neutralizing antibody production. With delivery systems for the NANP epitope apparently exhausted, many groups have switched focus to alternative delivery systems, such as DNA and powerful adjuvants. In concert with the identification of new pathogen-neutralizing B and T cell epitopes from other stages of the parasite lifecycle. While this work will increase the repertoire of reagents which can be used to develop effective malaria vaccines, we demonstrate that a delivery system that optimizes the interaction between the CS epitopes at hand, and the host immune system, could result in an effective malaria vaccine.

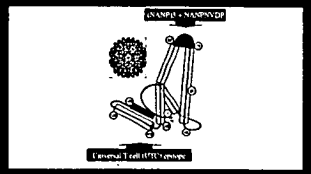
HBc as a Carrier of Heterologous Epitopes

The hepatitis B core protein is one of the more immunogenic proteins known. In its natural state it forms the core of the hepatitis B virus and the immune system of essentially every individual who becomes infected with the virus develops a powerful immune response to the core [4].

Recombinantly produced, hybrid hepatitis B core proteins have been shown to significantly enhance the immune response to inserted foreign sequences displayed at the surface with a conformation that mimics the structure of the sequence in the parent [5]. The variety of hybrids produced in this manner have proven HBc to be amazingly tolerant to the internal addition of foreign sequences at the site of the immunodominant loop, while retaining the ability to form particles that can be easily purified in a manner that is largely independent of the inserted sequence.

As a carrier moiety for B cell epitopes HBc offers other significant benefits. The particulate structure stimulates immunogenicity and allows incorporation of multiple epitope copies that are conformationally constrained. These features confer superior results relative to non-particulate carriers such as KLH, BSG or tetanus and diphtheria toxoids [6]. Further, HBc can directly activate B cells, elicit strong Th cell responses, and is efficiently processed and presented by antigen presenting cells (unpublished data).

Structure of MalariVax™



The B cell Epitope, (NANP)n

The immunodominant B cell epitope of the CS protein of *P.falciparum* is a highly conserved, repetitive tetrapeptide (NANP) [7], and antibodies to this epitope have been shown to be sporozoite neutralizing by protecting against rodent and human malaria. Immune responsiveness to this epitope has been positively correlated with immunity to malaria in both vaccine recipients and naturally infected individuals.

The Malaria Specific T cell Epitope, CS 329-345

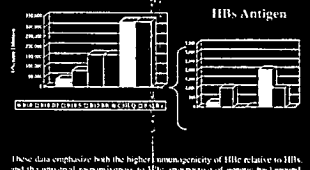
While the T cell help afforded by HBc is highly effective in enhancing antibody responses (i.e. B cell exclusive) to 'carnal' epitopes following vaccination, it will not activate malaria specific T cells. To ensure the priming of malaria specific T-helper cells as well as B cells, we have included a malaria specific T-helper epitope in our vaccine. The benefits of the inclusion of a universal Th cell epitope derived from the malaria parasite are several-fold. First, the priming of malaria specific Th cells ensures that, should a vaccine recipient be exposed to malaria, a more rapid and stronger anti-malaria response will be activated due to previous priming of malaria specific T-helper cells. Secondly, vaccinees living in malaria endemic regions will experience natural 'boosting' every time they are exposed to the parasite because their immune systems have been primed at both the B and Th cell level. This effect is similar to clinical boosting by re-vaccination - a process which would be difficult to enforce in third world countries where malaria is often endemic.

Nardin and coworkers at NYU recently identified a exogenous form of the T cell epitope CS 326-345 which appears to bind to all class II molecules [8]. Studies have shown that this exogenous epitope is 'universal', like the T cell help afforded by HBc, and suggests that it will prime malaria specific Th cells in essentially all vaccine recipients.

Antibody Responses

Like HBc, HBs is a particulate protein derived from the hepatitis B virus which has been proposed as a carrier for heterologous epitopes. We have studied the relative immunogenicity of HBcAg compared with HBcAg, and the ability of each to evoke immune responses in different genetic backgrounds [9]. Briefly, mice in the indicated strain were immunized with a single 20ug dose, IP, of either HBcAg or HBsAg and antibody titers against the immunogen determined via ELISA.

CorVax™ is Superior to the HBs Malaria Platform

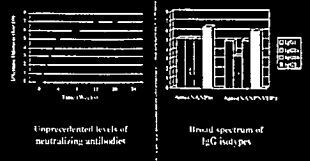


These data emphasize both the higher immunogenicity of HBc relative to HBs, and the universal responsiveness to HBc, irrespective of genetic background. For example, HBc is 5,000x more immunogenic than HBs in BALB/c mice, and, while both BDF1 and B10.S1 mice are non-responders for HBs, every strain tested is responsive to HBc. These results re-emphasize the suitability of HBc as a vaccine carrier and specifically its superiority over HBs, hence our selection of HBc as opposed to HBs as carry heterologous epitopes, and, in the case of malaria, avoidance of genetic restriction and inadequate antibody titers which have prevented the development of an effective vaccine.

The data below illustrates the breadth of appearance of significant titers of anti-CS antibodies and the broad spectrum of isotypes produced.

Preclinical Results: Antibody Response

MalariVax™ elicits high titer antibody that is specific for sporozoites and encompasses all IgG subtypes



Unprecedented levels of neutralizing antibodies

Broad spectrum of IgG Isotypes

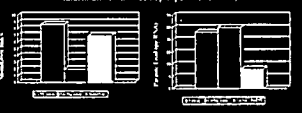
Cellular Responses

To test for the functionality of the CS-UTC epitope when presented on HBc particles to humans, human T cells from patients immunized with UTC containing peptides were exposed to either UTC peptide, HBcAg, or V12.PS3.1 which contains the UTC epitope fused to the C-terminus. The results of these experiments, shown in the left panel, confirm that human T cells proliferate when exposed to UTC containing particles, but not control particles. The importance of this experiment is that it demonstrated that the UTC epitope, delivered in the context of HBcAg, can efficiently stimulate human T cells.

To test the ability of HBc particles to present the UTC epitope in vivo, and also the effect of priming UTC specific T cells on malaria infection, a particle displaying only the UTC epitope from *P.yoelii* was synthesized. The results of this experiment revealed that mice immunized with Py-UTC containing particles exhibited a dramatic reduction in parasitic load in the liver, which was not observed in either non-immunized mice or mice receiving HBc particles that did not contain the Py-UTC epitope (Right Panel).

Preclinical Results: Cellular Response

Malaria universal T cell epitope is functional)



Human malaria-specific T cells recognize the malaria UTC epitope on MalariVax™

In *P.yoelii*, the UTC epitope delivered on CorVax™ reduced parasite load via CD4+ T cells

Left Panel - Human T cells from individuals immunized with UTC containing synthetic peptide immunogens were analyzed for proliferative responses upon exposure to V12.PS3.1, which contains the UTC epitope at the C-terminus of monomers.

Right Panel - Balb/c mice were immunized with 20ug of the indicated particles in CFA on day 1 and boosted with 20ug of the same particle in IFA on day 16. On day 44 mice were challenged with 50,000 live *P.yoelii* sporozoites. On day 48 the livers of mice were extracted and analyzed by RT-PCR for parasite specific RNA.

Using rodent models of malaria, ICC's founding scientists successfully demonstrated that malaria CS-repeats fused to the immunodominant loop of HBc were able to protect mice against both *P.berghei* and perhaps more impressively, *P.yoelii* to levels of 90-100% (Ho, IJ).

Preclinical Results: Animal Protection

Immunogen	IFA Titer	Protection
P. berghei CorVax™	40,960	95%
P.ynelii CorVax™	12,800	95%
P.falciparum CorVax™ (MalariVax™)	5,200.00	Not Tested

MalariVax™ is >100-fold More Potent than Protective Vaccines

Discussion

We have shown that our hepatitis B nucleocapsid derived *falciparum* malaria vaccine MalariVax™ is capable of inducing a powerful immune response to the CS-repeat epitopes of *P. falciparum*. It has long been recognized that antibodies to this repeat are protective against parasitic infection. In addition, we have shown that the universal T cell epitope from the CS protein can be incorporated in a way that makes it functional in the vaccine.

The HBcAg is currently immunogenic in chimpanzees after immunization and in humans during a natural infection. While toxicological studies of HBc-based products have not been conducted to date there is no evidence of toxicity due to HBc in HBV asymptomatic chronic carriers or in HBcAg-expressing transgenic mice.

MalariVax™ is produced in *E.coli* and purified in a process that is both rapid and scalable. We believe that we will be able to produce large amounts of vaccine for a cost that will make it practical for large scale immunization programs throughout the world.

Conclusions

- MalariVax™ is HBc to 1,000-fold more immunogenic than competing vaccines in small animals
- Genetic restriction is not seen when inbred strains of mice are immunized with MalariVax™
- Human malaria-specific T-cells recognize the malaria UTC epitope on MalariVax™
- In *P. yoelii*, the UTC epitope delivered on CorVax™ reduced parasite load by a CD4 cell dependent mechanism
- CorVax™ protects mice from infection with *Plasmodium berghei* and *P. yoelii*
- MalariVax™ is >100-fold more potent than protective rodent vaccines

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